leukocyte interferon interact differently with several of the individual purified leukocyte interferon species. All these observations lead us to postulate the existence of a mechanism that generates diversity in the primary structure of human leukocyte interferon.

Results

The first chromatographic step for the purification of camel pro-opiocortin was on Sephadex G-100. The high molecular weight region contained two peaks that showed opioid activity after digestion with trypsin, corresponding in molecular weights to pro-opiocortin (ca. 30,000) and β-lipotropin (10,000). Rechromatography on Sephadex G-75 was used to complete the separation of pro-opiocortin from β-lipotropin and other proteins of similar size. Reverse-phase chromatography resolved several major protein peaks, one of which corresponded with the opioid activity. The fraction having the highest specific activity (fraction 25) was rechromatographed under similar conditions, except for the collection of smaller fractions (0.75 ml) and the use of a lower flow rate (15 ml/hr). A single symmetrical peak was obtained. The fraction with the highest specific activity (fraction 50) was found to be homogeneous by polyacrylamide gel electrophoresis in NaDodSO$_4$. About 5 nmol of pure proopiocortin was isolated from the extract of one camel pituitary by this procedure. This represented a 77-fold purification from the initial acid extract with a 15% overall yield. Five other camel pituitaries were carried through the same procedure. Yields as high as 50% were obtained with these pituitaries by rechromatographing less pure fractions.

We claim:

1. A process for purifying proteins having molecular weights greater than about 12,000 which process comprises passing an aqueous solution of said protein in an impure state through a buffer equilibrated octyl bonded silica matrix column under high performance liquid chromatography conditions so as to absorb said protein onto said column and thereafter eluting said protein from said column with an increasing gradient of an aqueous water miscible solvent and obtaining said protein in selected fractions of said eluate in a state of enhanced purity.

2. A process for purifying proteins having molecular weights greater than about 12,000 which process comprises passing an aqueous solution of said protein in an impure state through a buffer equilibrated glyceryl bonded silica matrix column under high performance liquid chromatography conditions so as to absorb said protein onto said column and thereafter eluting said protein from said column with a decreasing gradient of an aqueous water miscible solvent and obtaining said protein in selected fractions of said eluate in a state of enhanced purity.

3. A process for producing interferon as a homogeneous protein which process comprises the following steps in combinations:

A. passing an aqueous solution of interferon in an impure state through a buffer equilibrated octyl bonded silica matrix column under high performance liquid chromatography conditions so as to absorb said interferon onto said column and thereafter eluting said interferon from said column with an increasing gradient of an aqueous, buffered, water miscible solvent and obtaining said interferon in selected fractions of said eluate in a state of enhanced purity;

B. passing the selected interferon fractions obtained in Step A through a buffer equilibrated glyceryl bonded silica matrix column to absorb said interferon onto said column and thereafter eluting said interferon from said column with a decreasing gradient of an aqueous buffered water miscible solvent and obtaining interferon as distinct major peaks in selected fractions of said eluate in a state of enhanced purity;

C. passing the selected fractions of interferon corresponding to one of said distinct major peaks obtained in Step B in buffered solution through a buffer equilibrated octyl bonded silica matrix column under high performance liquid chromatography conditions so as to absorb said interferon onto said column and thereafter eluting said interferon from said column with an aqueous, buffered, water miscible solvent and obtaining interferon as a single distinct peak in selected fractions of said eluate in the state of a homogeneous protein.

4. The process of claim **3** wherein said interferon is a human interferon.

5. The process of claim **4** wherein said human interferon is a human leukocyte interferon and the procedure of Step C is repeated to achieve ultimate homogeneity.

6. The process of claim **5** wherein said water miscible solvent is selected from an alkanol or a cyclic ether.

7. The process of claim **6** wherein said water miscible solvent is n-propanol, said buffer in Step A provides a pH of about 7.5, and said buffer in Step C provides a pH of about 4.0.

8. The process of claim **7** wherein said buffer in Step A is 1 M sodium acetate-acetic acid and said n-propanol gradient is increased from 0 to about 40% (v/v), said buffer in Step B is 0.1 M sodium acetate and said n-propanol gradient is decreased from about 72.5 to about 50% (v/v), said buffer in Step C was 1 M pyridine 2 M formic acid and said n-propanol gradient is increased from about 20 to about 40% (v/v).

9. The process of claim **8** wherein the selected peak fractions from Step B are pooled, the n-propanol removed by extraction with n-hexane and the traces of n-hexane are removed from the aqueous phase prior to proceeding to Step C.

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